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Research Article

Assessing the diversity of nematodes in the Store Mosse National Park (Sweden) using metabarcoding

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Abstract

The Store Mosse National Park in the south of Sweden was surveyed for nematode diversity and distribution using DNA metabarcoding. Fifty samples were collected from five vegetation types in the park across a range of habitats (e.g. soil, litter, lichens, sphagnum and roots). The other habitats, aside from soil and litter, were sampled in order to capture the diversity of nematodes that may be uniquely associated with them. Nematodes were characterised using the V7-V8 variable domain (~ 350 bp) of the 18S ribosomal RNA gene. We identified 46 families, 76 genera (21 new to Swedish fauna) and 60 species (31 new to Swedish fauna). Some nematodes showed strong associations with their habitats, especially at the species level. Although soil and litter supported the most diverse nematode communities, our results support a strong justification for sampling across different media types to quantify nematode diversity accurately. Soil and litter communities showed high levels of stability with balanced distribution of all the various trophic and coloniser-persister groups.

Key words: litter, molecular marker, national park, Nematoda, soil, vegetation

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Introduction

An estimated 15% of the total land area of Sweden is protected. National parks are afforded the strongest form of protection and Sweden has 30 of them, including Store Mosse. Store Mosse attained the status of a national park in 1982 as one of the largest bog complexes in southern Sweden (Martínez Cortizas et al. 2021) covering approximately 7,682 hectares. The largest component of the park is in the form of wetlands known to support rich plant and animal life. The park consists mostly of raised bog, open swamps and fens (Naturvårdsverket 2015). Standing between 160 and 170 m above sea level, the area records an annual average of +6 °C temperature and 766 mm precipitation (Kylander et al. 2013). The peat layers covering its raised bogs are believed to have accumulated over a period of nearly 10,000 years (Ryberg et al. 2022) and, hence, are considered a paleoarchive (Martínez Cortizas et al. 2021). However, aside from the paleoenvironmental studies assessing the impacts of changes in vegetational

composition on the biological and chemical structure of the soil (Kylander et al. 2013; Martínez Cortizas et al. 2021; Ryberg et al. 2022), very little work has been done on a biodiversity inventory. With the exception of Stenmark (2020) providing a list of disturbance-sensitive vascular plant species and Jentzsch and Steinborn (2009) a list of dipteran species, not much else is known.

Nematodes represent a species-rich group that occurs across a wide range of habitats with astonishing abundance (Holterman et al. 2006; van den Hoogen et al. 2019). Great morphological, genomic and functional diversity exists amongst nematodes, allowing them to play diverse roles in ecosystem functioning. As a result of this, nematodes have been used as effective biological indicators for assessing the conditions of their environment (Vanaverbeke et al. 2004; Höss et al. 2011). For example, high abundance of sensitive taxa associated with later successional stages in a sample is typically indicative of a complex community structure and, hence, a matured and stable condition (Bongers 1990). Originally, nematode identification was based on morphology – a time-consuming task that requires specialised expertise. Molecular identification methods provide faster and often more accurate alternatives to morphological identification, require less taxonomic expertise and can easily be automated (Blok 2005; Ahmed et al. 2016). The first amongst these methods was DNA barcoding, which involves the use of a targeted DNA region for discriminating species (Floyd et al. 2002; Blaxter 2003; Hebert et al. 2003; Blaxter et al. 2005), but remained constrained by its throughput (Creer et al. 2010). This limitation has been alleviated by the advent of next-generation sequencing and its application in metabarcoding (Porazinska et al. 2009; Creer et al. 2010; Taberlet et al. 2012). This approach has revolutionised how we tackle questions related to biodiversity assessment (Guardiola et al. 2015) across many fields of faunistic studies in nematodes (Porazinska et al. 2010a; Bik et al. 2012). Within nematology, most of the earlier studies on metabarcoding sought to evaluate the performance of different genomic regions and to establish robust pipelines for analysing bulk nematode samples (Porazinska et al. 2009; Creer et al. 2010; Ahmed et al. 2019). Porazinska et al. (2009) assessed the suitability of metabarcoding for nematode community analysis and established a benchmark for future nematode metabarcoding studies. Other studies have also focused on developing alternative PCR and sequencing primers that provide broader taxonomic coverage and better species resolution (Sapkota and Nicolaisen 2015; Waeyenberge et al. 2019; Kenmotsu et al. 2020; Sidker et al. 2020; Kawanobe et al. 2021). Similar benchmarking studies have been carried out on nematodes in freshwater and marine habitats (Holovachov et al. 2017; Macheriotou et al. 2019; Schenk et al. 2020).

In a practical sense, metabarcoding contributed to a better understanding of nematode diversity across different terrestrial environments, as well as habitat types other than the commonly-analysed mineral fraction of the soil or litter. This is particularly important because such habitats often harbour some unique diversity of nematodes that are seldom found in the mineral component of the soil alone. For example, Porazinska et al. (2010b) were amongst the first to apply metabarcoding to study the diversity of nematodes within soil, litter and canopy of the Costa Rican rainforest. They concluded that nematode diversity was potentially higher in tropical regions than in temperate regions, albeit

not necessarily in soil, contrasting a previously-held notion that suggested the contrary. As a follow-up to this study, the main authors published another paper where they demonstrated that nematode species richness in the tropical rainforest was three times more than it is in temperate rainforest, thus further supporting their earlier finding (Porazinska et al. 2012). In their contribution to understanding the latitudinal differences in the diversity of nematodes, Kerfahi et al. (2016) also used a metagenetic approach to examine nematodes from the tropical rainforest of Malaysia and the Arctic tundra of Svalbard. They observed no difference in diversity between the two ecosystems. The fact that only soil samples were used in their analysis may explain this observed difference with Porazinska et al. (2010b, 2012).

We used metabarcoding to reveal the extent of nematode diversity within the Store Mosse National Park (Sweden). We sampled across five vegetation types. Additionally, we collected not just mineral soil, but other habitat types such as litter, lichens, sphagnum, roots, decomposed wood, moss, fungus and samples from below anthills. We hypothesise that sampling strategies restricted to the soil habitat underestimate nematode diversity and sampling across habitat types is more critical than across vegetation types. Moreover, Stenmark (2020) identified a number of sensitive plants in his species list, suggesting that the area has not been exposed to much disturbance. Therefore, we hypothesise that a similar observation will be made regarding nematodes, in that there will be a good number of sensitive late successional stage nematode taxa present.

Material and methods

Study site, sample collection and processing

All samples were collected on the 13 and 14 of October 2021 within the Store Mosse National Park (160–170 m a.s.l.) located in the county of Jönköping in southern Sweden (Fig. 1). Samples were collected from 50 spots across all vegetation types present in the Park (Figs 1, 2, Suppl. material 1) and consisted of nine different types of habitats (Table 1) to capture nematode diversity beyond the commonly-sampled mineral soil. Some types of vegetation were more heavily sampled than others, because they were easily accessible. To minimise the impact of sampling on undisturbed environments, all samples were collected in close proximity to roads and hiking trails. Whenever possible, samples were collected using a corer with inner diameter of 16 mm, collecting 100 ml for each sample. Lichen, fungi and moss samples were collected by hand. Decomposed wood samples were collected by hand or by scraping them off with a pocket knife. Samples were then stored at 6 °C until extraction. Extraction was carried out in batches of 10 samples at a time. The last set of samples was processed within four weeks of collection. To facilitate nematode isolation, dense samples were manually disintegrated prior to extraction. Nematodes were extracted from 100 ml of media using the Whitehead tray method (Whitehead and Hemming 1965) for 48 hours. The nematode extracts were concentrated in microcentrifuge ending up with 50 µl of suspension.

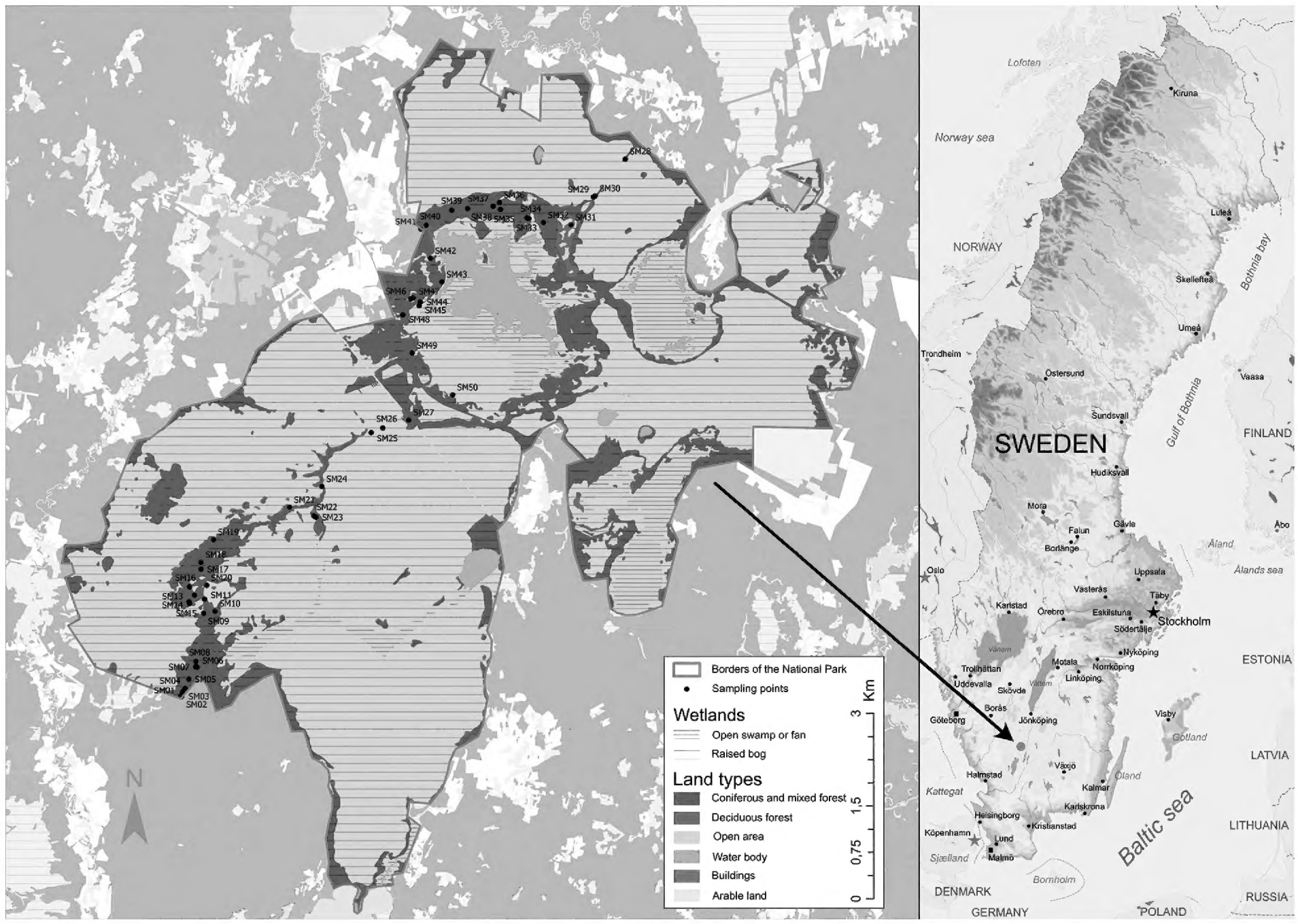

Figure 1. Map of Store Mosse National Park (Sweden) showing sampling locations and the different vegetation types.

Table 1. Summary of sampled vegetation types and habitats.

Habitat (No of samples)	Vegetation				
	Coniferous forest on dry land	**Coniferous forest on raised bog**	**Deciduous forest**	**Raised bog**	**Open swamp and fen**
Soil (11)	SM5, SM6, SM20, SM27	SM8, SM33, SM46, SM47	SM31, SM40		SM42
Litter (18)	SM1, SM2, SM3, SM4, SM10, SM12, SM16, SM24, SM38	SM11, SM19, SM22, SM28, SM32		SM25, SM26, SM29, SM50	
Decomposed wood (6)		SM15, SM18, SM36		SM30, SM45, SM49	
Moss (4)	SM23, SM48	SM35		SM44	
Fungus (3)	SM17, SM39, SM43				
Lichens (3)	SM7, SM14		SM41		
Anthill (2)	SM37	SM34			
Sphagnum (2)	SM21			SM9	
Roots (1)	SM13				

DNA Extraction, PCR and NGS

Genomic DNA extraction was performed on each sample using the Qiagen QiAmp DNA Micro kit. Briefly, 130 µl of ATL buffer was added to each sample (concentrated to 50 µl), followed by 20 µl of proteinase K. Tissue lysis and DNA purification were performed following the manufacturer's instructions. We used 18S rRNA

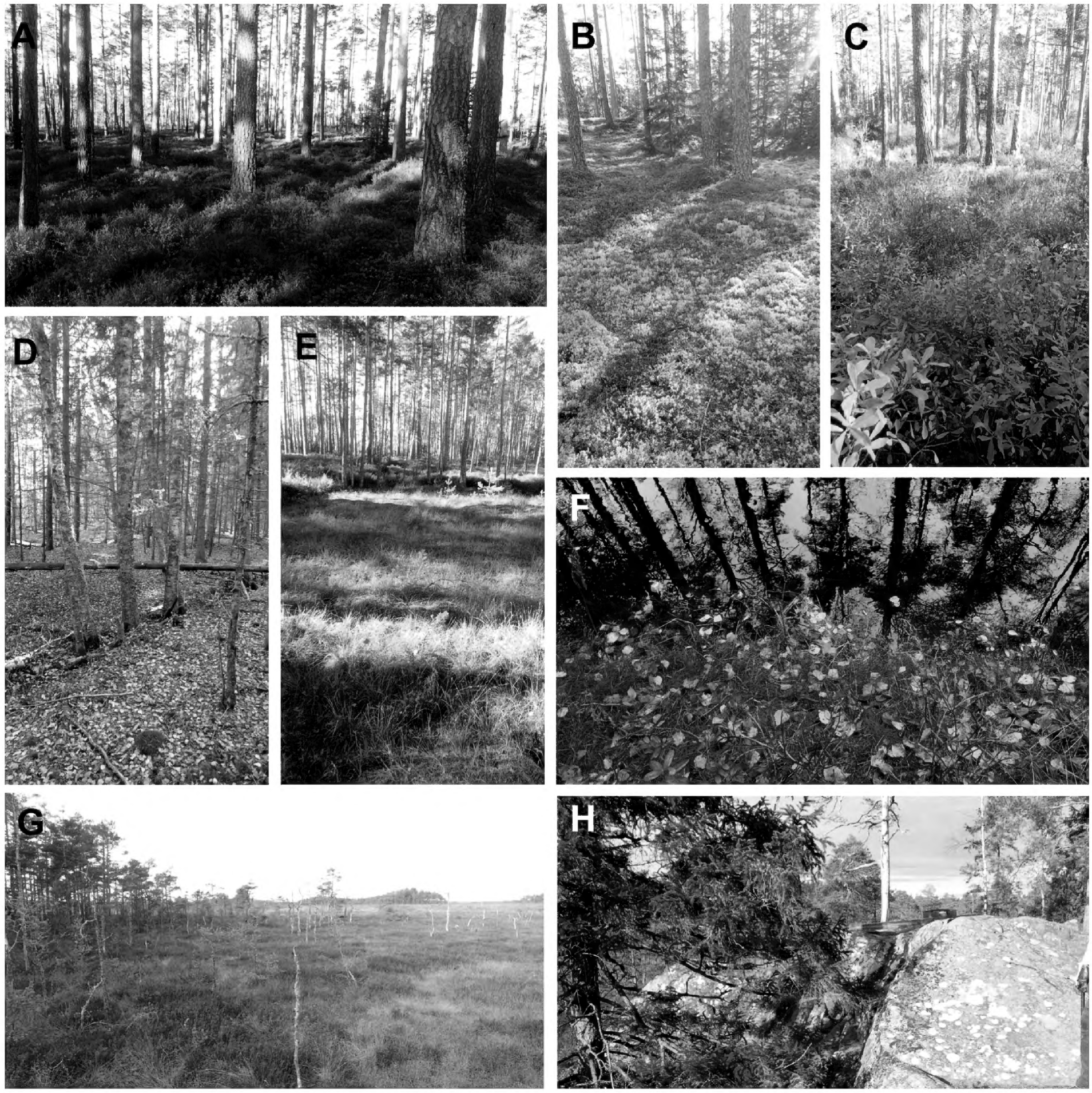

Figure 2. Images showing the different sampling locations. **A** Lingonberries vegetation under a pine forest. **B** Pine forest with lichen and moss ground cover. **C** Pine forest with lingonberries, *Sphagnum* and other bushes as ground cover. **D** Fir and birch forest with litter covering the ground surface. **E** Open area within a pine forest covered with sedge ground cover. **F** The bank of an artificial channel within a coniferous forest. **G** Grassland with lower sphagnum cover. **H** Granite outcrops with lichen and moss.

metabarcoding with the NF1 (5'-GGTGGTGCATGGCCGTTCTTAGTT-3', matching the 5' end of the 38th helix) and 18Sr2b (5'-TACAAAGGGCAGGGACGTAAT-3', matching the 3' end of the 32nd helix) primers (Porazinska et al. 2009) extended by Illumina adapters to amplify the V7-V8 domain (~ 350 bp). Three technical replicates of the DNA extracts from each sample were used for both the initial and index PCR. Amplification was performed in a 25 µl reaction mixture using Illustra Hot Start Mix RTG 0.2 ml reaction kit (GE Healthcare Life Sciences, Sweden). The reaction mixture consisted of 1 µl (0.4 µM) of each primer, 2 µl of template DNA and 21 µl of nuclease-free water. The cycle conditions set were as previously

described in Ahmed et al. (2019). Following the index PCR, the triplicates were reconstituted into one before purification. The amplicons were purified using Agencourt AMPure XP (Beckman Coulter, California, United States) and sent to Macrogen Europe B.V. (Amsterdam, The Netherlands) for library preparation and sequencing using Illumina MiSeq 2×300 bp paired-end sequencing. Raw data are available at NCBI Sequence Read Archive under the BioProject ID PRJNA923582.

Bioinformatics

Analysis of the raw sequencing data was performed using a 64-bit USEARCH v.11.0.667 (Edgar 2010). Forward and reverse reads were merged using the *-fastq_mergepairs* (options: overlap set to > 150 bp, < 10 mismatches within the overlapping region and > 80% identity in the overlapping region). Merged reads were filtered using the *-fastq_filter* (options: maximum expected error per sequence set to 1, minimum length of the sequences set to 250). Using the *-fastx_uniques*, the remaining reads were dereplicated and then clustered using the UNOISE algorithm, as implemented in the *-unoise3*, to obtain zero-radius operational taxonomic units (ZOTUs) (Edgar 2016) equivalent to amplicon sequence variants (ASVs). For consistency with other studies, we will refer to ZOTUs as ASVs. All parameters for the clustering command were set to default values including a *-minsize* value of 8 to retain ASVs with at least 8 reads. The usearch command *-otutab* was used to create an ASV table. Using the *-sintax* command and the PR2 database version 4.14.0 (Guillou et al. 2013), the ASVs were assigned taxonomy. Only assignments with posterior probability scores of ≥ 0.8 were considered. A pattern-matching script was used to extract ASVs matching Nematoda from the sintax taxonomy assignment. These ASVs were then searched for and extracted from the ASV sequence files and ASV table. The extracted nematode ASVs were again assigned taxonomy, this time using a custom high-quality nematodes-only curated database with more accurate taxonomies, based on the classification of De Ley and Blaxter (2004). Initially, the high posterior probability score cut-off set for the sintax taxonomy resulted in ASVs associated with Dorylaimidae and Qudsianematidae failing to be assigned to the correct families. BLAST search results were, therefore, used to complement the sintax taxonomy. A phylogenetic tree of the nematodes was generated for MAFFT-aligned (Katoh and Standley 2013) ASVs using FastTree (Price et al. 2010). All parameters were left at their default settings for MAFFT alignment and, for FastTree analysis, the 'gtr' model was used. The ASV table, taxonomy file, phylogenetic tree and metadata file (e.g. vegetation type, habitat and soil type) were exported into R for statistical analyses.

Statistical analyses

To test our hypothesis that mineral soil will account for only a portion of nematode diversity, we compared nematode communities across different habitats. All analyses were carried out using R version 4.0.5 (R Core Team 2021) inside RStudio (RStudio Team 2022). Abundance information was based on sequence read counts for all ASVs representing each taxon in a sample. Richness was computed, based on Chao1 diversity index using the phyloseq package of McMurdie and Holmes (2013). Significant differences in Richness between soil and litter were analysed using Wilcoxon test (Wilcoxon 1945). In addition,

differences in nematode community compositions across different habitats were demonstrated using non-metric multidimensional scaling (NMDS) ordination of Bray-Curtis dissimilarity metrics. Most of the analysis involving NMDS were performed following the workflow described in Ji et al. (2013). The function *vegdist* of the vegan package (Oksanen et al. 2015) was used to calculate distances; and *metaMDS* for performing NMDS. The statistical significance of community composition was evaluated using PERMANOVA with the vegan package function *adonis2*. Pairwise comparisons of the nematode communities across the different habitats were performed using *betadisper.TukeyHSD*.

To test our hypothesis that there will be a good representation of sensitive nematodes in the Store Mosse National Park, we placed all taxa into trophic and coloniser-persister (c-p) groups using NINJA (Sieriebriennikov et al. 2014; https://shiny.wur.nl/ninja/), an online tool for nematode faunal analyses. The trophic groups were assigned following Yeates et al. (1993). The c-p groups were assigned a value 1 to 5, based on their r and K characteristics (Bongers 1990; Ferris et al. 2001) with taxa characterised by rapid growth, high fecundity, large gonads and small offsprings assigned low c-p, whereas taxa with slow development, large-bodies and fewer, but larger offspring (Neher 2010) with high c-p values. Colonisers dominate in communities that are heavily enriched or disturbed. In contrast, persisters tend to be indicative of pristine undisturbed conditions. Taxa not recognised by NINJA were replaced by their closest relative acceptable to NINJA or, where possible, replaced by a higher-ranking taxon (e.g. Basilaphelenchus replaced by Aphelenchoididae).

Results

General statistics

A total of 7,040,489 paired reads were generated. On average 85% of the paired reads were successfully merged per sample. Following filtering, 5,943,062 reads were retained. Clustering resulted in a total of 2,569 ASVs. The sintax algorithm assigned 31.8% of the ASVs (899 in total) to Nematoda (with posterior probability scores of ≥ 0.8), 18.7% were unassigned and the remainder were assigned to other eukaryotic lineages (Suppl. material 2) including tardigrades, platyhelminths, rotifers and arthropods.

Nematode diversity

We recovered a total of 46 nematode families with Tylenchidae representing the highest proportion (17.7%) of nematode ASVs (Suppl. material 3). At the genus level, the percentage of nematode ASVs with well-supported assigned taxonomy averaged ~ 60% and ranged from 7% to 96%. Amongst the 46 families recovered, we identified 76 genera (Table 2). The most represented families were Tylenchidae (11 genera), followed by Aphelenchoididae (7 genera) and Rhabditidae (8 genera), but most families were represented by a single genus. For 46 genera, at least some taxa could be resolved to the species level resulting in a total of 60 tentative nematode species. *Aphelenchoides* and *Malenchus* were the most diverse genera with four species associated with both. Amongst these 60 identified species, 31 are new to the fauna of Sweden (Dyntaxa 2023), see Table 2. Similarly, 21 genera have not been reported in Sweden until now (Dyntaxa 2023), see Table 2.

Table 2. List of genera and species of nematodes identified across all samples. The families for which genus assignment could not be achieved are not represented in this table. The number of ASVs identified for each taxon is given in parentheses. Genera and species new to the fauna of Sweden are underlined.

Trophic group	Family	Genus	Species
Bacterivore	Desmodoridae (86)	*Prodesmodora* (86)	
	Monhysteridae (61)	*Eumonhystera* (59)	*Eumonhystera filiformis* (3)
		Geomonhystera (2)	
	Plectidae (34)	*Plectus* (25)	*Plectus minimus* (1), *P. tenuis* (6)
		Tylocephalus (2)	*Tylocephalus auriculatus* (1)
	Metateratocephalidae (21)	*Metateratocephalus* (16)	*Metateratocephalus crassidens* (7)
		Euteratocephalus (2)	*Euteratocephalus palustris* (1)
	Rhabditidae (20)	*Rhabditis* (10)	
		Poikilolaimus (2)	
		Choriorhabditis (1)	*Choriorhabditis lacustris* (1)
		Diploscapter (1)	*Diploscapter coronatus* (1)
		Oscheius (1)	*Oscheius dolichura* (1)
		Pellioditis (1)	
		Protorhabditis (1)	
	Panagrolaimidae (13)	*Panagrolaimus* (7)	
		Baldwinema (1)	*Baldwinema ardabilense* (1)
	Alaimidae (12)	*Alaimus* (4)	*Alaimus parvus* (1)
	Xyalidae (10)	*Theristus* (9)	*Theristus agilis* (8)
	Aphanolaimidae (9)	*Aphanolaimus* (4)	*Aphanolaimus aquaticus* (2)
	Prismatolaimidae (8)	*Prismatolaimus* (8)	*Prismatolaimus dolichurus* (3)
	Teratocephalidae (7)	*Teratocephalus* (7)	*Teratocephalus deconincki* (2)
	Cephalobidae (6)	*Acrobeloides* (4)	*Acrobeloides varius* (1)
	Chronogastridae (6)	*Chronogaster* (6)	
	Bunonematidae (5)	*Bunonema* (5)	*Bunonema reticulatum* (1), *B. richtersi* (1)
	Rhabdolaimidae (3)	*Rhabdolaimus* (3)	
	Alloionematidae (1)	*Rhabditophanes* (1)	
	Diplopeltidae (1)	*Cylindrolaimus* (1)	
	Ethmolaimidae (1)	*Ethmolaimus* (1)	*Ethmolaimus pratensis* (1)
Fungivore	Diphtherophodridae (8)	*Tylolaimophorus* (5)	*Tylolaimophorus typicus* (5)
		Diphtherophora (1)	
Fungivore / Herbivore	Tylenchidae (151)	*Malenchus* (68)	*Malenchus acarayensis* (4), *M. bryanti* (3), *M. neosulcus* (17), *M. pressulus* (6)
		Miculenchus (24)	*Miculenchus muscus* (5)
		Filenchus (16)	*Filenchus facultativus* (7), *F. misellus* (1)
		Tylenchus (9)	*Tylenchus arcuatus* (2), *T. naranensis* (1)
		Ecphyadophora (7)	*Ecphyadophora tenuissima* (2)
		Irantylenchus (4)	*Irantylenchus vicinus* (2)
		Cephalenchus (3)	*Cephalenchus hexalineatus* (2)
		Aglenchus (1)	*Aglenchus agricola* (1)
		Basiria (1)	
		Coslenchus (1)	*Coslenchus costatus* (1)
		Discotylenchus (1)	

Trophic group	Family	Genus	Species
Fungivore / Herbivore	Aphelenchoididae (115)	*Aphelenchoides* (68)	*Aphelenchoides blastophthorus* (1), *A. heidelbergi* (5), *A. ritzemabosi* (3), *A. saprophilus* (1)
		Laimaphelenchus (16)	*Laimaphelenchus penardi* (6)
		Basilaphelenchus (15)	
		Potensaphelenchus (3)	*Potensaphelenchus stammeri* (1)
		Ektaphelenchoides (2)	
		Bursaphelenchus (1)	
		Schistonchus (1)	
	Anguinidae (25)	*Anguina* (4)	
		Ditylenchus (14)	*Ditylenchus adasi* (1), *D. destructor* (1)
	Sphaerulariidae (5)	*Paurodontella* (2)	*Paurodontella gilanica* (2)
		Veleshkinema (2)	*Veleshkinema iranicum* (2)
	Neotylenchidae (4)	*Hexatylus* (4)	*Hexatylus viviparus* (3)
Herbivore	Hoplolaimidae (2)	*Helicotylenchus* (1)	*Helicotylenchus pseudorobustus* (1)
	Tylenchulidae (2)	*Paratylenchus* (2)	
	Pratylenchidae (1)	*Pratylenchus* (1)	*Pratylenchus crenatus* (1)
	Telotylenchidae (1)	*Tylenchorhynchus* (2)	*Tylenchorhynchus parvulus* (2)
		Neodolichorhynchus (1)	
	Trichodoridae (1)	*Paratrichodorus* (1)	*Paratrichodorus pachydermus* (1)
Omnivore	Aporcelaimidae (11)	*Aporcelaimellus* (9)	*Aporcelaimellus obtusicaudatus* (7)
	Nordiidae (11)	*Enchodelus* (5)	
		Pungentus (1)	
	Tylencholaimidae (8)	*Tylencholaimus* (6)	*Tylencholaimus mirabilis* (3), *T. teres* (1), *T. zhongshanensis* (1)
	Nygolaimidae (6)	*Paravulvus* (2)	*Paravulvus hartingii* (2)
	Dorylaimidae (5)	*Crassolabium* (1)	*Crassolabium circuliferum* (1)
		Prodorylaimus (1)	
	Actinolaimidae (2)	*Paractinolaimus* (1)	
Predator	Mononchidae (25)	*Clarkus* (3)	*Clarkus papillatus* (2)
		Mononchus (10)	*Mononchus truncatus* (7)
		Prionchulus (9)	*Prionchulus muscorum* (9)
	Diplogastridae (5)	*Pristionchus* (3)	
	Tripylidae (3)	*Tripyla* (2)	
	Mylonchulidae (1)	*Mylonchulus* (1)	
Zooparasitic / zoopathogenic	Angiostomatidae (1)	*Angiostoma* (1)	*Angiostoma norvegicum* (1)
	Heterorhabditidae (1)	*Heterorhabditis* (1)	
	Steinernematidae (1)	*Steinernema* (1)	*Steinernema kraussei* (1)

Nematode communities across habitats and vegetation

Soil and litter supported the highest richness. The two habitats showed comparable richness (Fig. 3). Compared to the other habitats combined, both soil and litter were significantly richer. In contrast, fungus, lichens and decomposing wood showed low diversity. The two most heavily sampled vegetation, coniferous forest on dry land and coniferous forest on raised bog showed a wide range of diversity across sampled spots. Between them, there was a significant difference in their alpha diversity (Suppl. material 4).

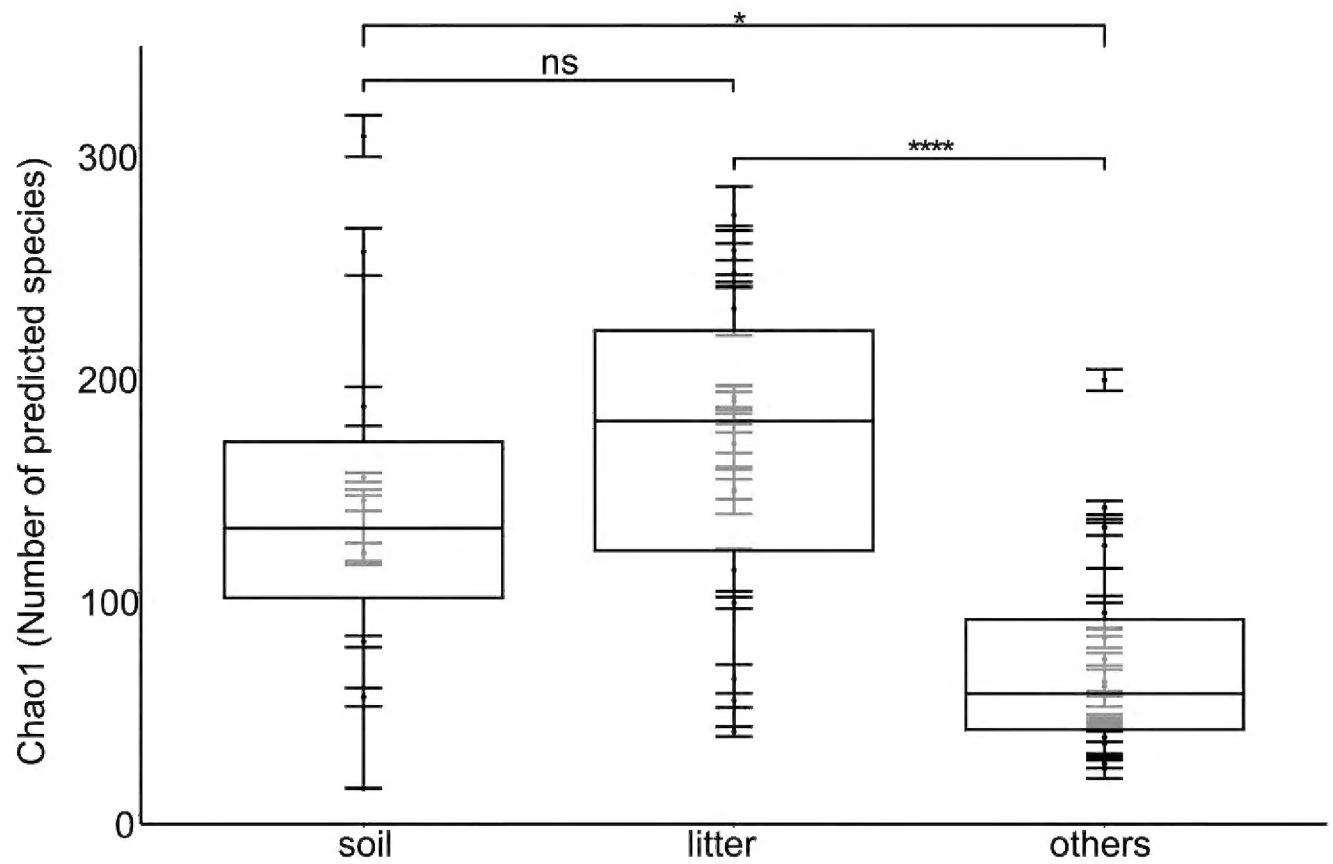

Figure 3. Chao1 measures the α-diversity of nematodes in different habitats. The group "others" represent all the other habitats combined. Statistical significance of the difference between alpha diversity measures were tested using the Wilcoxon test. ns = p > 0.05 (not significant); * = p <= 0.05; **** = p <= 0.0001.

Amongst the bacterivores and zooparasites, reads associated with Plectidae, Metateratocephalidae and Rhabditidae were the most dominant across all habitats (Fig. 4a). The soil samples had representations for all three families. One sample (SM46) comprised almost entirely of Rhabditidae with another sample (SM47) having no reads for bacterivores and zooparasites. Litter samples, on the other hand, comprised mostly of reads associated with Plectidae and Metateratocephalidae. The litter samples also showed a pattern of taxonomic composition that appeared more similar to one another than the soil samples did. Wood samples were also dominated by these three main families, Plectidae, Metateratocephalidae and Rhabditidae. Moss and lichen were dominated by reads associated with Plectidae. Two of the three fungus samples were dominated by reads associated with Alloionematidae, while one comprised mainly of Cephalobidae and Rhabditidae. Amongst fungivores and herbivores, reads associated with Tylenchidae and Diphtherophoridae dominated both soil and litter samples (Fig. 4b). Reads associated with Anguinidae were also found in high abundance in some soil samples. Litter samples were similar to soil samples in their taxonomic composition, with the exception of one sample that comprised almost entirely of Neotylenchidae. Wood, moss fungus, lichen and anthill comprised mostly of Aphelenchoididae. *Sphagnum* and root were dominated by Tylenchidae. Of the taxa belonging to omnivores and predators, Qudsianematidae were the most dominant, followed by Mononchidae and Tylencholaimidae (Fig. 4c). Although not dominant in any of the samples they occurred, Nordiidae were second in terms of prevalence only to Qudsianematidae. Nematode distribution across samples within the same vegetation types did not show the same level of uniformity observed in samples from the same habitats (Suppl. material 5).

In terms of prevalence, most taxa showed wide distribution across multiple habitats and vegetation (Suppl. materials 6, 7). *Rhabditis* (soil), *Poikilolaimus* (wood), some species of *Basilaphelenchus* (wood) and an unidentified

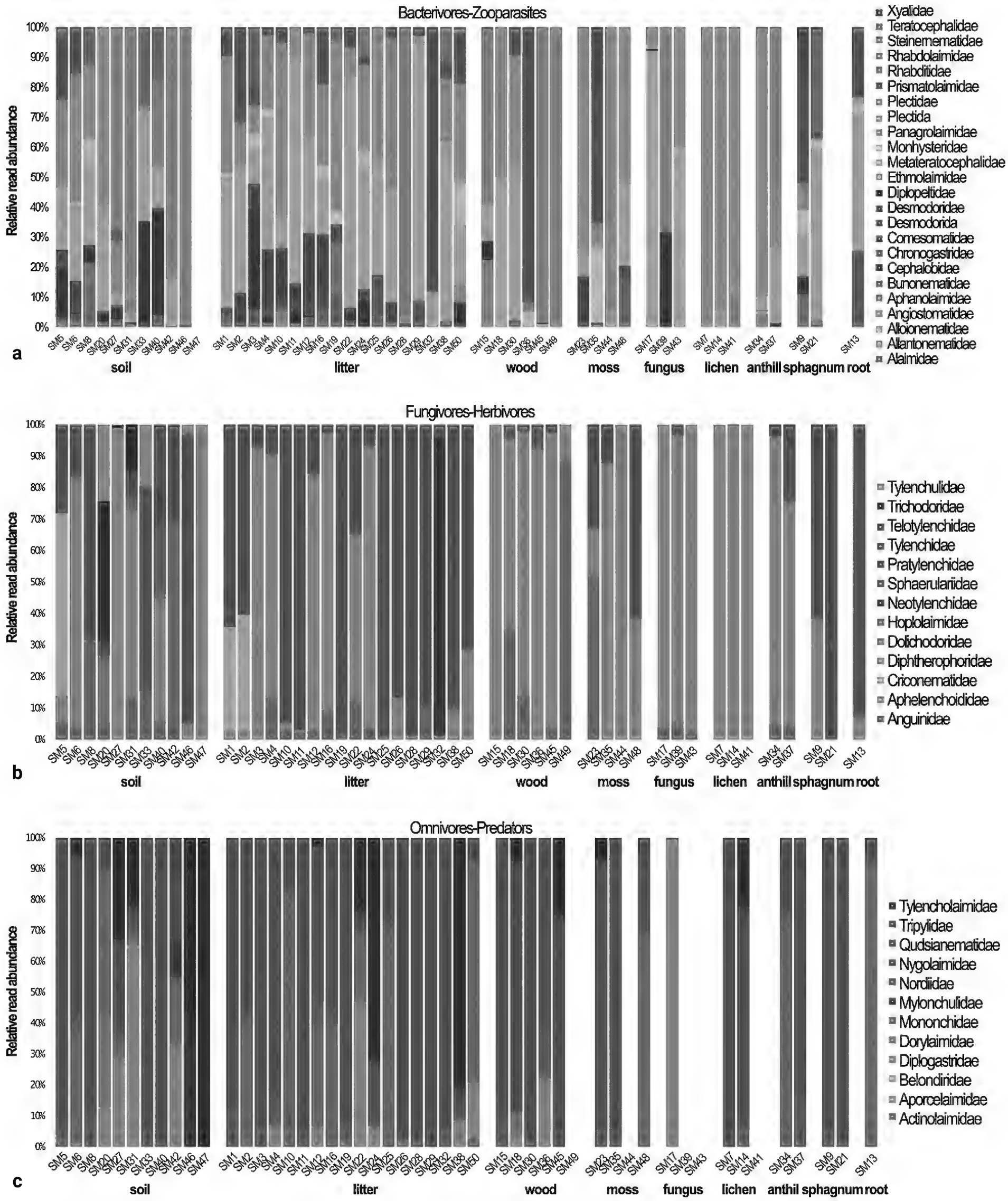

Figure 4. Read distribution amongst nematode families. Each bar corresponds to a sample. Samples are aggregated into various habitat types. Not all taxa were resolved to the species level. These are represented at the order or class rank.

Dorylaimida (soil) were confined to only one habitat. *Heterorhabditis* (coniferous forest on dry land), Criconematidae (coniferous forest on dry land), some unidentified species of Dorylaimida (coniferous forest on raised bog) and unidentified Triplonchida (coniferous forest on dry land) were also associated with just one vegetation type.

Nematode communities amongst sampled media

Differences between nematode communities across the habitats as depicted by NMDS ordinations showed a clear separation (Fig. 5) ($F = 1.75$, $P < 0.001$). Pairwise comparison revealed that soil samples were significantly different from anthill, *Sphagnum* and root samples. With respect to litter, only root and *Sphagnum* were significantly different. Although wood samples appeared different from all other samples (Fig. 5), they were only significantly different from anthill, roots and *Sphagnum*. Across the vegetation types, NMDS plots showed no differences (Suppl. material 8).

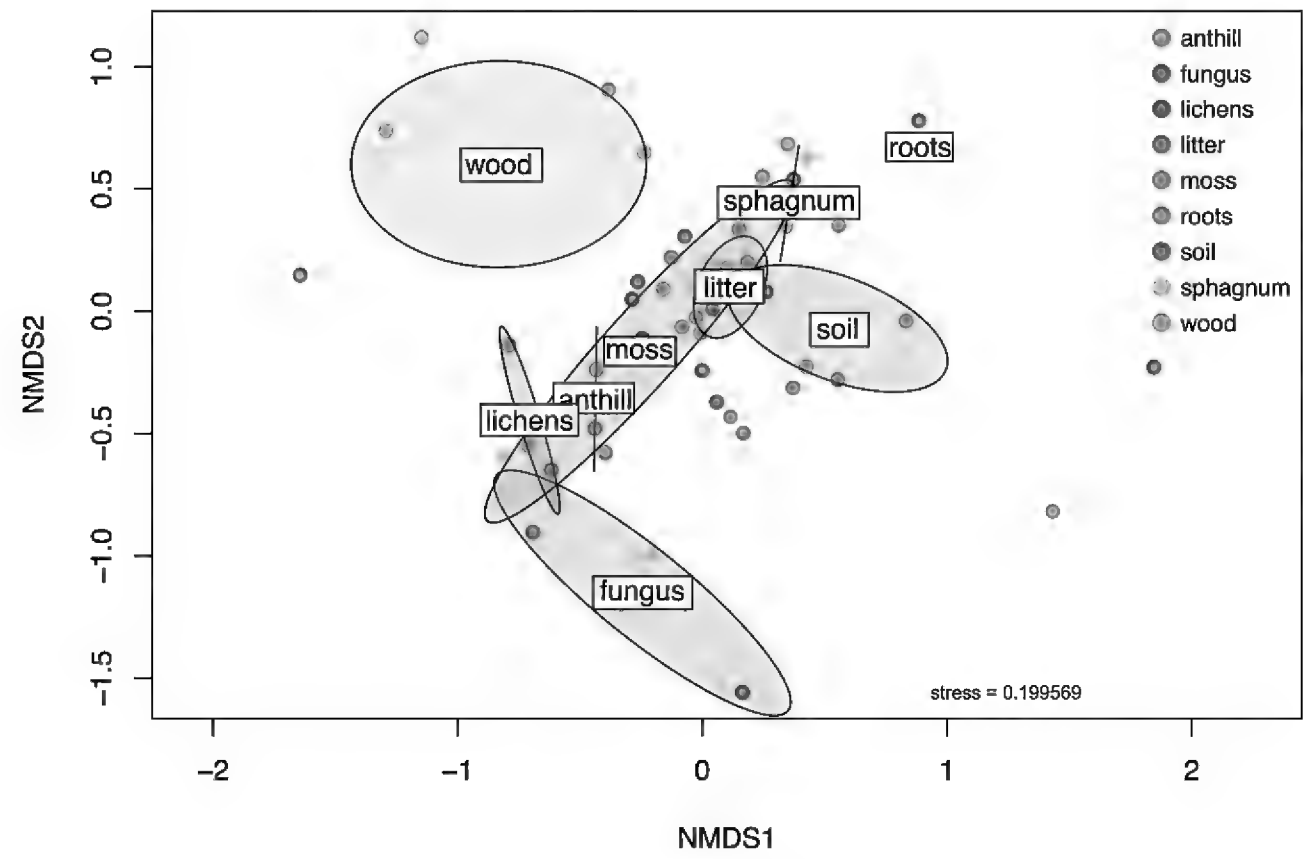

Figure 5. Non-metric multidimensional scaling (NMDS) ordinations. Points are individual samples and coloured ellipses are 95% confidence intervals of species centroids for each habitat (ellipses generated using the function, 'ordiellipses' inside vegan).

Analysis of disturbance levels and food web in the communities

Using the c-p triangle to depict the stability/enrichment/stress conditions of the communities, most samples appeared to be in good stable conditions (Fig. 6a, b). The soil and litter samples, where most of the diversity occurred, showed the highest stability, while at the same time showing very low levels of enrichment. Wood and lichen samples were generally depicted as stressed, except for a few samples of decomposing wood. The two anthill samples were in low stability states.

Based on the interpretation by Ferris et al. (2001), most of the samples regardless of the habitat were either in a matured or maturing food web state (Fig. 6b). Lichen was the only habitat for which all samples were in a degraded, depleted state. Soil and litter samples were mostly concentrated within the high structure quadrants (maturing to matured food web), albeit with varying degrees of enrichment. Fungus samples showed low maturity and appeared to be highly disturbed in some samples and enriched in others. The wood samples varied greatly in the states of the food webs they depicted, while some appeared matured and fertile/N-enriched, others were highly disturbed and moderate to heavily enriched. The two anthill samples were in quite opposite conditions, one in a maturing state, while the other was in a degraded and nutrient-depleted state.

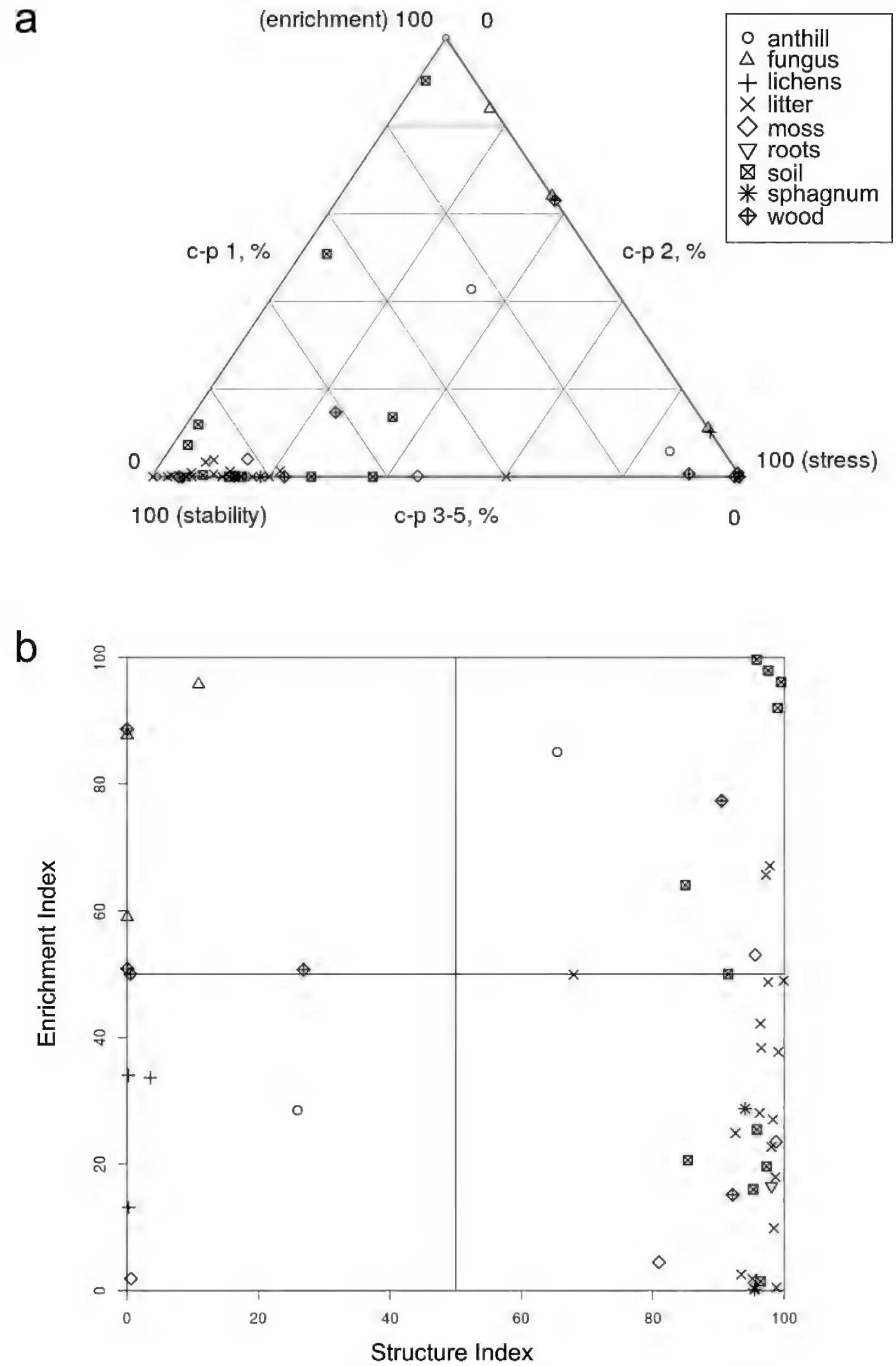

Figure 6. c-p triangle and food web analysis of the different habitats. c-p triangles (**a**) depict the stability of the communities. Food web analysis plots (**b**) depict the maturity of the food webs within the communities. **a** c-p triangle showing samples categorised under different habitats. **b** Food web analysis showing samples categorised under different habitats.

Association between nematode species and habitat

The sample-species network showed that several species were exclusively associated with specific media types (Suppl. materials 9, 10). Aside from some plant-feeding nematodes that were expected to be, to some degree, only associated with soil samples, species such as *Alaimus parvus*, *Choriorhabditis*

cristata, *Clarkus papillatus* were also only detected in the soil samples. *Hexatylus viviparus*, *Ethmolaimus pratensis*, *Mononchus truncatus*, *Bunonema richtersi* and *Steinernema kraussei* were exclusively associated with litter. *Ektaphelenchoides spondylis*, *Potensaphelenchus stammeri* and *Angiostoma margaretae* were also only detected in wood (Suppl. material 10).

Discussion

Our analysis recovered a massive diversity of nematodes, with a total of 46 nematode families across 10 different orders. We identified a total of 76 nematode genera and, within 46 of these, we identified taxa to a species level. Soil and litter were the two most sampled habitats and most of their samples shared similar compositions at the family level. However, some soil samples were distinct by being dominated by Rhabditidae, which were not observed at such high abundance in the litter samples. The other habitats, on the other hand, harboured unique nematode communities, confirming our initial hypothesis. Our findings show that sampling solely mineral soil would miss 25% of taxa at the species level, underscoring the necessity of sampling diverse habitats as demonstrated by previous authors (Powers et al. 2009; Porazinska et al. 2010b, 2012). Although collection of samples beyond soils has been evaluated and practised in the past (Yeates 1972; Sohlenius and Boström 2001), most biodiversity studies still focus only on soil samples. This must be taken into consideration when attempting to create baseline metabarcoding datasets for different biomes to be used as reference points in the future monitoring of ecosystem changes. The general recommendation is to include all habitats where nematodes can occur, including, but not limited to, bodies or other organisms (plants, fungi and animals, motile and immotile).

Despite our inability to identify the qudsianematid ASVs beyond the family level, the recovery of 76 genera was remarkable, especially that this study was limited to a single sampling event. In comparison with other regions in Sweden or similar climatic conditions, the Store Mosse National Park clearly shows significantly higher nematode diversity. Specifically, the Scots pine forest in Sweden sampled three times over the course of 25 years (156 total soil samples) supported only 36 unique nematode taxa (Sohlenius and Boström 2001). Although the majority of identified genera were also recovered in this study, we detected over 50 additional genera. Likewise, a study of a mire in northern Sweden, sampled over a period of four months, identified 24 taxa representing 17 unique genera (Sohlenius et al. 1997). Similarly, an 85-year-old Danish beech forest studied over a period of 12 months was characterised by the presence of only 41 genera (Yeates 1972). One major reason for this disparity is the diversity of habitat types analysed in our study. Another factor could be associated with methods used to identify nematodes. Unlike morphology, metabarcoding can identify not only immature individuals, but also eggs (Gendron et al. 2022).

Nematode composition showed no association with vegetation type and instead was influenced more by the habitat. Across the different types of vegetation, none showed any unique pattern of nematode distribution at the family level. Due to the strong influence the habitat has on the community, a better comparison of communities under the different vegetation types would be one

that is restricted to only one type of habitat. However, a comparison of the vegetation types for only soil samples also showed no significant influence of vegetation type on the nematode community.

Indices used in this study that describe the structure and maturity of the community are heavily dependent on abundance data. Moreover, since sequence read abundance does not directly correlate with the abundance of taxa in a typical metabarcoding analysis, there is constraint in the inferences that can be made about the condition of the samples, based on these indices (Waeyenberge et al. 2019). Nevertheless, the largely stable conditions depicted by our analysis confirm the pristine nature of the Store Mosse National Park. The depiction of fungus samples as showing low maturing and high level of disturbance could be indicative of a decomposition pathway that is more fungal-driven than bacterial-driven. The states of the food web across wood samples were highly varied. A possible explanation for this could be the fact that wood decomposition is a complex multistage process that includes different organisms during different times, with wood at late stages becoming similar to litter and soil.

In conclusion, our analyses have shown the close and sometimes exclusive association between certain taxa and medium types, highlighting the pertinence of sampling across multiple habitats/media. According to the food web analysis and c-p triangles, most of the samples, irrespective of the habitat, were in stable undisturbed states. We also identified several new taxa records for the Swedish forest. The use of metabarcoding was key in achieving the level of taxonomic resolution observed in this study.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No approval from the Swedish Ethical Review Authority is required.

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Author contributions

MA and OH concieved a study design, collected and analysed the data. DS provided a reference database. All authors contributed to the writing of the manuscript and approved the final version prior to its submission.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Sampling data

Authors: Mohammed Ahmed, Dieter Slos, Oleksandr Holovachov

Data type: doc

Link: https://doi.org/10.3897/mbmg.8.111307.suppl1

Supplementary material 2

Relative abundance of ASVs

Authors: Mohammed Ahmed, Dieter Slos, Oleksandr Holovachov

Data type: jpg

Explanation note: Relative abundance of ASVs associated with major groups of eukaryotes in all 50 samples combined, including ASVs that were not assigned to any group of eukaryotes.

Link: https://doi.org/10.3897/mbmg.8.111307.suppl2

Supplementary material 3

Proportions of total ASVs assigned to various nematode families including those unassigned at the family level across all samples

Authors: Mohammed Ahmed, Dieter Slos, Oleksandr Holovachov

Data type: jpg

Link: https://doi.org/10.3897/mbmg.8.111307.suppl3

Supplementary material 4

Vegetation types

Authors: Mohammed Ahmed, Dieter Slos, Oleksandr Holovachov

Data type: jpg

Explanation note: Number of samples representing each type of vegetation included in parenthesis. Statistical significance of the difference between alpha diversity for Coniferous forest on dry land and Coniferous forest on raised bog samples was tested using the Wilcoxon test. * = significant.

Link: https://doi.org/10.3897/mbmg.8.111307.suppl4

Supplementary material 5

Read distribution amongst nematode families across the different types of vegetation

Authors: Mohammed Ahmed, Dieter Slos, Oleksandr Holovachov

Data type: jpg

Explanation note: Each bar corresponds to a sample. Samples are aggregated into various vegetation types.

Link: https://doi.org/10.3897/mbmg.8.111307.suppl5

Supplementary material 6

Maximum Likelihood tree of the 100 most dominant ASVs

Authors: Mohammed Ahmed, Dieter Slos, Oleksandr Holovachov

Data type: jpg

Explanation note: Maximum Likelihood tree of the 100 most dominant ASVs showing their prevalence and abundance across different samples and habitat/medium types. Leaf nodes are labelled with the assigned taxa (genus where possible) of the ASVs. Circles represent the samples and the diameters of the circles indicate the abundance of the taxon in samples. Circles of the same colour indicate samples from the same habitat/medium type.

Link: https://doi.org/10.3897/mbmg.8.111307.suppl6

Supplementary material 7

Maximum Likelihood tree of the 100 most dominant ASVs

Authors: Mohammed Ahmed, Dieter Slos, Oleksandr Holovachov

Data type: jpg

Explanation note: Maximum Likelihood tree of the 100 most dominant ASVs showing their prevalence and abundance across different samples and vegetation types. Leaf nodes are labelled with the assigned taxa (genus where possible) of the ASVs. Circles represent the samples and the diameters of the circles indicate the abundance of the taxon in samples. Circles of the same colour indicate samples from the same vegetation type. Con. = Coniferous, Dec. = Deciduous.

Link: https://doi.org/10.3897/mbmg.8.111307.suppl7

Supplementary material 8

Non-metric multidimensional scaling (NMDS) ordinations

Authors: Mohammed Ahmed, Dieter Slos, Oleksandr Holovachov

Data type: jpg

Explanation note: Points are individual samples and coloured ellipses are 95% confidence intervals of species centroids for each type of vegetation (ellipses generated using the function, 'ordiellipses' inside vegan).

Link: https://doi.org/10.3897/mbmg.8.111307.suppl8

Supplementary material 9

NMDS plots of samples and taxa at the species level

Authors: Mohammed Ahmed, Dieter Slos, Oleksandr Holovachov

Data type: jpg

Explanation note: Samples are coloured based on habitat. Acr_var = *Acrobeloides varius*, Agl_agr = *Aglenchus agricola*, Ala_par = *Alaimus parvus*, Ang_mar = *Angiostoma margaretae*, Aph_aqu = *Aphanolaimus aquaticus*, Aph_bla = *Aphelenchoides blastophthorus*, Aph_hei = *Aphelenchoides heidelbergi*, Aph_rit = *Aphelenchoides ritzemabosi*, Aph_sap = *Aphelenchoides saprophilus*, Apo_obt = *Aporcelaimellus obtusicaudatus*, Bal_ard = *Baldwinema ardabilense*, Bun_ret = *Bunonema reticulatum*, Bun_ric = *Bunonema richtersi*, Cep_hex = *Cephalenchus hexalineatus*, Cho_cri = *Choriorhabditis cristata*, Cla_pap = *Clarkus papillatus*, Cos_cos = *Coslenchus costatus*, Cra_cir = *Crassolabium circuliferum*, Dip_cor = *Diploscapter coronatus*, Dit_ada = *Ditylenchus_adasi*, Dit_des = *Ditylenchus_destructor*, Ecp_ten = *Ecphyadophora tenuissima*, Ekt_spo = *Ektaphelenchoides spondylis*, Eth_pra = *Ethmolaimus_pratensis*, Eum_fil = *Eumonhystera filiformis*, Eut_pal = *Euteratocephalus palustris*, Fil_fac = *Filenchus facultativus*, Fil_mis = *Filenchus misellus*, Hel_pse = *Helicotylenchus pseudorobustus*, Hex_viv = *Hexatylus viviparus*, Ira_vic = *Irantylenchus vicinus*, Lai_pen = *Laimaphelenchus penardi*, Mal_aca = *Malenchus acarayensis*, Mal_bry = *Malenchus bryanti*, Mal_neo = *Malenchus neosulcus*, Mal_pre = *Malenchus pressulus*, Met_cra = *Metateratocephalus crassidens*, Mic_mus = *Miculenchus muscus*, Mon_tru = *Mononchus truncatus*, Osc_dol = *Oscheius dolichura*, Par_pac = *Paratrichodorus pachydermus*, Par_har = *Paravulvus hartingii*, Pau_gil = *Paurodontella gilanica*, Ple_min = *Plectus minimus*, Ple_ten = *Plectus tenuis*, Pot_sta = *Potensaphelenchus stammeri*, Pra_cre = *Pratylenchus crenatus*, Pri_mus = *Prionchulus muscorum*, Pri_dol = *Prismatolaimus dolichurus*, Ste_kra = *Steinernema kraussei*, Ter_dec = *Teratocephalus deconincki*, The_agi = *Theristus agilis*, Tyl_mir = *Tylencholaimus mirabilis*, Tyl_ter = *Tylencholaimus teres*, Tyl_zho = *Tylencholaimus zhongshanensis*, Tyl_par = *Tylenchorhynchus _parvulus*, Tyl_arc = *Tylenchus arcuatus*, Tyl_nar = *Tylenchus naranensis*, Tyl_aur = *Tylocephalus auriculatus*, Tyl_typ = *Tylolaimophorus typicus*, Vel_ira = *Veleshkinema iranicum*.

Link: https://doi.org/10.3897/mbmg.8.111307.suppl9

Supplementary material 10

Species network showing the association between taxa and samples

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Data type: jpg

Explanation note: Samples are represented by hexagonal nodes; taxa are represented by circular light beige nodes. Sample-taxon associations are depicted by edges (arrowed lines) extending from the sample to the taxon. The shorter the edge between a sample and a taxon, the more abundant the taxon is in the sample. Habitats are represented by different colours. Both the nodes representing a sample and the edge (arrowed line) extending from it are coloured to depict the habitat to which it belongs. Taxa located at the periphery of the network are, in most cases, the ones detected in only one type of habitat, whereas those at or near the centre of the network are found in multiple samples.

Link: https://doi.org/10.3897/mbmg.8.111307.suppl10